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13. ABSTRACT *(Maximum 200 words)*

In recent studies we demonstrated that pretreatment of hamsters with specific 5-HT receptor agonists, or the serotonin (5-HT) precursor, tryptophan, markedly attenuated a variety of light-induced responses of the SCN clock, These included immediate-early gene (Fos) activation in SCN cells, light-mediated SCN field potentials, phase-shifts of the free-running circadian activity rhythm and phase advances of the light-entrained activity rhythm. Based upon these clear and repeatable effects of 5-HT in the SCN, it was hypothesized that 5-HT modulates the effect of light on the timing of circadian physiological and behavioral rhythms. The central findings of the funded studies were that endogenous 5-HT released from terminals within the SCN has physiological roles in: 1) modulating the strength of the photic entraining signal relayed to the SCN from the retina and 2) eliciting phase-shifts in the activity of the circadian clock. These results point to a potentially powerful means of pharmacologically manipulating the human circadian system as a strategy for ameliorating circadian-related disfunctions.

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RATIONALE AND HYPOTHESES

Although 5-HT is known to play an important role in circadian system function (Honma et al., 1979; Wirz-Justice et al., 1982; Tamarkin et al., 1983; Banky et al., 1988; Morin et al., 1990; Prosser et al., 1990; Albers et al., 1991a; Tominaga et al., 1992), its biological role in SCN pacemaker function is not well understood. A growing body of evidence suggests that 5-HT may take part in the entrainment of circadian rhythms by the LD cycle (Nishino and Kiozumi, 1977; Shibata et al., 1983; Miller and Fuller, 1990). The principal hypothesis to be examined was that 5-HT acts to modulate the SCN pacemaker's response to light. The effects of treatment with various serotonergic drugs on SCN responses to light were investigated to test for this hypothesis. Expression of Fos was employed as a biological marker to study activation of SCN cells by light as in other studies (Colwell et al., 1990; Abe et al., 1991 and 1992).

SPECIFIC AIMS

Experiment 1: The initial aim of experiment 1 was to examine the modulatory effects of the non-selective serotonergic agonist, quipazine on light-induced Fos-LI in the SCN during the late subjective night.

Experiment 2: The aim of experiment 2 was to examine the modulatory effects of specific serotonergic agonists on photic

induction of Fos-LI in the SCN, in order to characterize the 5-HT receptor type/subtype(s) that mediate 5-HT actions in the SCN. The hypothesis to be tested is that 5-HT actions in the circadian pacemaker are mediated via a specific 5-HT receptor-linked mechanism.

Experiment 3: The initial aim of experiment 3 was to investigate the modulatory effects of drugs, known to enhance *in vivo* 5-HT synthesis, on light-induced Fos-LI. The hypothesis to be tested is that endogenous 5-HT modulates photic signalling to the SCN. The second aim of this experiment was to determine the 5-HT receptor(s) involved in circadian pacemaker function.

Experiment 4: The aim of experiment 4 was to examine a possible effect of melatonin on light-induced Fos-LI in the SCN during the late subjective night. The hypothesis to be tested is that the modulatory effects of 5-HT on photically induced Fos-LI is mediated via melatonin-dependent mechanism(s).

Experiment 5: The main objective of experiment 5 was to examine the effects of L-tryptophan, the immediate 5-HT precursor, on entrainment of the circadian activity rhythm by the LD cycle. The hypothesis to be tested is that the modulatory effect of 5-HT on light-induced Fos-LI in the SCN is important with regard to expression of overt circadian

rhythms.

Experiment 6: The main goal of experiment 6 was to examine the effects of different serotonergic agonists and antagonists on induction of SCN Fos-LI in response to phase delaying light stimulation (during the early subjective night). The hypothesis to be tested is that a time-dependent differential 5-HT-mediated modulation of Fos expression in the SCN may underlie the phase specific effects of light on the circadian oscillator.

Choice of the animal species

The Syrian hamster (*Mesocricetus Auratus*) was used in the present study for a variety of reasons. (1) It is photoperiodic and it has been used as an animal model in circadian research (Daan and Pittendrigh, 1974; Rusak, 1979; Prosser et al., 1992 and 1993; Tominaga et al., 1992; Edgar et al., 1993). (2) Several studies have investigated and characterized many of the neurochemical aspects of 5-HT in the SCN of this species (Glass et al., 1992; Prosser et al., 1992 and 1993; Srkalovic et al., 1994). (3) Photo-inducibility and immunocytochemistry of c-Fos have been studied extensively in this species (Earnest et al., 1990; Kornhauser et al., 1990; Abe et al., 1991; Rea et al., 1991 and 1992).

SIGNIFICANCE

Results from this study will help elucidate the biological role of 5-HT and the receptor mechanism(s) underlying its actions in the circadian pacemaker. Such information would provide new insights to researching the neurochemical mechanisms involved in the responses of the mammalian circadian system to light. It would also bring into account new approaches to the management of chronopathologies and optimizing performance during periods of desynchronization of the circadian system. The potential advantage of rapid resynchronization of the circadian system is important with regard to human health, performance and welfare.

MATERIALS AND METHODS

GENERAL CONSIDERATIONS

Animals

Adult Syrian hamsters (*Mesocricetus Auratus*) of both sexes ranging in age from 8 to 15 weeks and weighing 125-150 grams were used in this study. Animals were maintained under a 14L:10D photoperiod with light intensity of approximately 120 lux, in temperature-controlled chambers (25°C). Food and water were provided ad libitum. One week prior to experimental trials, animals were housed singly in clear plastic cages, some equipped with running wheels.

C-Fos immunocytochemistry

Animals were prepared for Fos immunocytochemistry as follows: animals were given a lethal dose of Nembutal(0.8 ml/animal)and were perfused intracardially with 100 ml of Zamboni's buffered picric acid paraformaldehyde fixative (75 ml saturated aqueous solution of picric acid, 75 ml deionized H_2O, 18.0 g paraformaldehyde, 2 M NaOH and 850 ml phosphate buffer [pH=7.3]). Brains were removed, blocked at the anterior and posterior borders of the hypothalamus and post-fixed in Zamboni's fixative overnight at 4°C. Eight serial 75

μm-thick vibratome sections, containing the mid-posterior extent of the SCN, were collected. Sections were washed twice with 0.5 M Tris buffered saline (TBS) (8.10 g Nacl, 900 ml deionized H_2O, 1.0 g bovine serum albumin and 60.6 g Tris in 500 ml deionized H_2O [pH=7.6]) with 0.1% BSA, 0.01 merthiolate and 0.1% Triton X-100 and then incubated in TBS containing 1.0% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity. Sections were again rinsed twice with TBS and then incubated for 24 hours at 4°C with a commercially-available rabbit polyclonal primary C-Fos antibody (Oncogene Science), against the N-terminal peptide 4-17 residues of human Fos protein, and diluted at a concentration of 1:750 in TBS containing 0.1% BSA, 0.1% Triton 100 and 0.01% merthiolate. The tissue was then washed two more times with TBS containing 0.1% BSA and incubated with a biotinylated rabbit secondary antibody (Elite-ABC kit; vector labs) diluted in TBS, for 60 minutes at room temperature. The tissue was then rinsed with TBS and incubated for one hour at room temperature with avidin-biotinylated peroxidase complex (Elite-ABC kit) diluted in TBS. After multiple rinses with 0.5 M TBS, sections were stained with a solution of 3,3-diaminobenzidine tetrahydrochloride (DAB) (0.2 mg/ml), 0.3% hydrogen peroxide and 0.05 M TBS for a period of 3-5 minutes. Staining was microscopically confirmed before the reaction was terminated by washing in 0.025 M TBS. Sections were then wet-

mounted on coated slides, dehydrated (air dry) and counter-stained with methylene blue for microscopic examination . Fos protein was detected as a brown reaction product in cell nuclei. The specificity of Fos-IR was verified by omission of the primary antibody in some sections. A standard immunocytochemical protocol was used and tissues were processed in large groups of 12-16 brains to minimize variability.

Drugs

Water-soluble drugs were dissolved in isotonic 0.9% NaCl (saline). Metergoline, L-tryptophan, ritanserin and 8-OH-DPAT were warmed gently in 20% DMSO in saline until completely dissolved.

Assessment of locomotor activity

An overhead infrared detector focused on the running wheel was used to monitor the animals' wheel-running activity. Output from the detector was integrated using a DATAQUEST III software (Minimitter) interfaced with an IBM-compatible computer. Wheel revolutions were counted in three minute bins. Twenty four-hour actograms were plotted horizontally from left to right, using the corrections described by Mrosovsky and Janik (1991).

EXPERIMENTAL PROTOCOLS

Drug injection: All drugs were administered by

intraperitoneal injection (i.p.). Injection volumes were 0.7 - 1.0 ml, and injections during the dark phase were administered with the aid of an infrared viewer and dim red illumination (<0.4 lux; Kodak #2 Wratten filter).

Experiment 1: The purpose of experiment 1 was to examine the effects of treatment with the non-specific serotonergic, quipazine on induction of SCN Fos-LI in response to a phase advancing light pulse. Hamsters received i.p. injections of either 0 (n=6), 2.5 (n=4), 5 (n=4) or 12.5 (n=6) mg/kg quipazine , administered 30 minutes prior to a 30 minute-duration light pulse (intensity = 120 lux) delivered at zeitgeber time [ZT] 19 (ZT 12 = lights-off and ZT 00 = lights-on). After termination of light stimulation, the animals were deeply anesthetized (Nembutal; 90 mg/kg) and processed for Fos immunocytochemistry as described above. Similarly, some vehicle-injected animals (n=4) were not exposed to light, but were sacrificed in the dark at the same time as the light-exposed animals.

Experiment 2: The purpose of experiment 2 was to determine the 5-HT receptor type/subtype(s) that mediate the effects of 5-HT on the SCN. Hamsters received an i.p. injection of either the 5-HT_{1A} receptor agonists, 8-OH-DPAT (0, 0.25, 1.0, 2.5 and 5 mg/kg) or buspirone (20 mg/kg), the 5-HT_{1B} receptor agonist, TFMPP (5 mg/kg), the 5-HT_2 receptor agonist, α-methyl

serotonin (5 mg/kg), the 5-HT_3 receptor agonist, 1-phenyl-biguanide (5 mg/kg), or the 5-HT_{1A} receptor antagonist, NAN-190 (10 mg/kg). An additional group of animals were treated with NAN-190 (10 mg/kg) 15 minutes before the injection of 8-OH-DPAT (5 mg/kg). Four animals were used at each dosage level in all treatments. Drugs were delivered at ZT 18.5, 30 minutes before the delivery of a light flash. Half an hour after the onset of light stimulation, animals were killed and their brains removed and processed for Fos immunocytochemistry.

Experiment 3: The aim of this experiment was to evaluate the effects of the endogenously released 5-HT on photic induction of Fos expression in the SCN and to determine the 5-HT receptor mechanism(s) mediating these responses. Hamsters received an i.p. injection of either a mixture of L-tryptophan (100 mg/kg), fenfluramine (7.5 mg/kg) and harmaline (10 mg/kg; n=4) 30 minutes prior to a 30 minute-duration light pulse (120 lux), or L-tryptophan alone (0; 150 mg/kg; each n=4) an hour prior to photic stimulation (7 hours after lights-off). The dose-responsiveness of L-tryptophan effects was examined in hamsters pretreated with L-tryptophan (0; n=6, 50; n=4, 100; n=4 and 200 mg/kg; n=5) 60 minutes before photic stimulation (intensity = 75 lux), at ZT 19. In order to characterize the 5-HT receptor(s) mediating tryptophan-induced responses, animals were treated with either NAN-190 (10 mg/kg; n=7), the

non-specific 5-HT antagonist, metergoline (10 mg/kg; n=4) or the $5\text{-}HT_{2/1C}$ receptor antagonist, ritanserin (10 mg/kg; n=4) 15 minutes before L-tryptophan loading (200 mg/kg). Additional groups of animals were treated with either metergoline (10 mg/kg; n=3) or ritanserin (10 mg/kg; n=3) alone, 30 minutes before delivery of the light pulse (75 lux) at ZT 19. Half an hour after the onset of light stimulation, animals were killed and their brains processed for Fos immunocytochemistry.

Experiment 4: The purpose of experiment 4 was to investigate whether melatonin mediates the effects of tryptophan-loading on light-induced Fos-LI in the SCN. Hamsters received an i.p. injection of melatonin (100 mg/kg) or vehicle (each n=4) 30 minutes prior to a 30 minute-duration light pulse applied at ZT 19. Half an hour after light stimulation, animals were sacrificed and their brains processed for Fos immunocytochemistry.

Experiment 5: The purpose of this experiment was to examine the effects of tryptophan-loading on entrainment of the circadian activity rhythms by the LD cycle. Wheel-running activity rhythms of hamsters maintained under a 14L:10D photoperiod were monitored for 7 days before, during and after pretreatment with i.p. injections of 150 mg/kg L-tryptophan (n=10) or vehicle (n=7) at ZT 21 (one hour before lights-on) for 5 consecutive days.

Experiment 6: The purpose of this experiment was to evaluate the effects of various serotonergic drugs on light-induced Fos-LI in the SCN during the early subjective night. Hamsters pretreated with an i.p. injection of either normal saline (n=8), quipazine (12.5 mg/kg), 8-OH-DPAT (5 mg/kg), NAN-190 (10 mg/kg) or metergoline (10 mg/kg) were exposed to a 30 minute-duration light flash (120 lux) delivered at ZT 13 (one hour after lights-off). Half an hour after light stimulation, animals were killed and their brains processed for Fos immunocytochemistry.

Statistical analysis

Fos experiments. The number of Fos-IR cells in the SCN was counted bilaterally in two consecutive sections, at comparable rostral-caudal level, that contained the largest number of stained nuclei (Abe et al., 1992). The mean count per section was calculated for each animal and the overall counts were expressed as the overall group mean $\pm$ SEM (Mead et al., 1992). Effects of drug treatments on the number of SCN cells expressing Fos-LI were standardized by expressing the final values as a percentage of the vehicle-control group mean. Analysis of variance (ANOVA) followed by a multiple range test (Student-Newman-Keuls) was used to test the differences between drug treatments on Fos-LI. The level for statistical significance was $p<0.05$.

Analysis of wheel-running behavior. Phase-shifts in activity rhythms were determined by visual inspection of double plotted actograms and measuring the difference between eye-fitted lines connecting the onset of activity (Colwell et al., 1993). Mean activity onset for each animal was calculated from 7-10 days immediately preceding tryptophan injections. Data were analyzed for statistical significance using the student t-test, $p<0.05$.

RESULTS

Effects of photic stimulation (ZT 19) on Fos-LI in the SCN

Exposure of control (vehicle-treated) hamsters to a 30 minute-duration light pulse delivered during the late subjective night (ZT 19) elicited a pattern of Fos-LI similar to that reported in previous studies in this species (Earnest et al., 1990; Abe et al., 1991 and 1992; Rea et al., 1992). The majority of cells displaying Fos-LI were localized to the ventrolateral, retino-receptive region of the caudal SCN (Rusak et al., 1990b; Rea et al., 1992; Figure 4A,B). Few immunoreactive cells were observed in the dorsolateral SCN and periventricular hypothalamic region. Scattered labeled cells were seen throughout the thalamus, piriform cortex and septal regions. Stained cells also were observed in the supraoptic nuclei (SON) of hamsters that received an i.p. injection containing normal saline. This expression of Fos-LI was not inhibited by any of the drugs employed and served as an internal control for the quality of the immunocytochemical procedures in each assay.

None of the animals treated with a vehicle and left in darkness exhibited Fos-LI in any region of the SCN. Few Fos-LI cells were seen in other brain regions, including the SON of the hypothalamus (data not shown).

Behavioral effects of serotonergic drugs

Animals treated with the selective 5-HT_{1A} agonist, 8-OH-DPAT exhibited some components of the previously described "5-HT behavioral syndrome" (Jacobs, 1976; Tricklebank et al., 1984; Curzon, 1990), in particular flat body posture and head weaving. Administration of either L-tryptophan, quipazine, buspirone and/or harmaline and fenfluramine also induced the same response, but to a lesser degree.

Experiment 1:

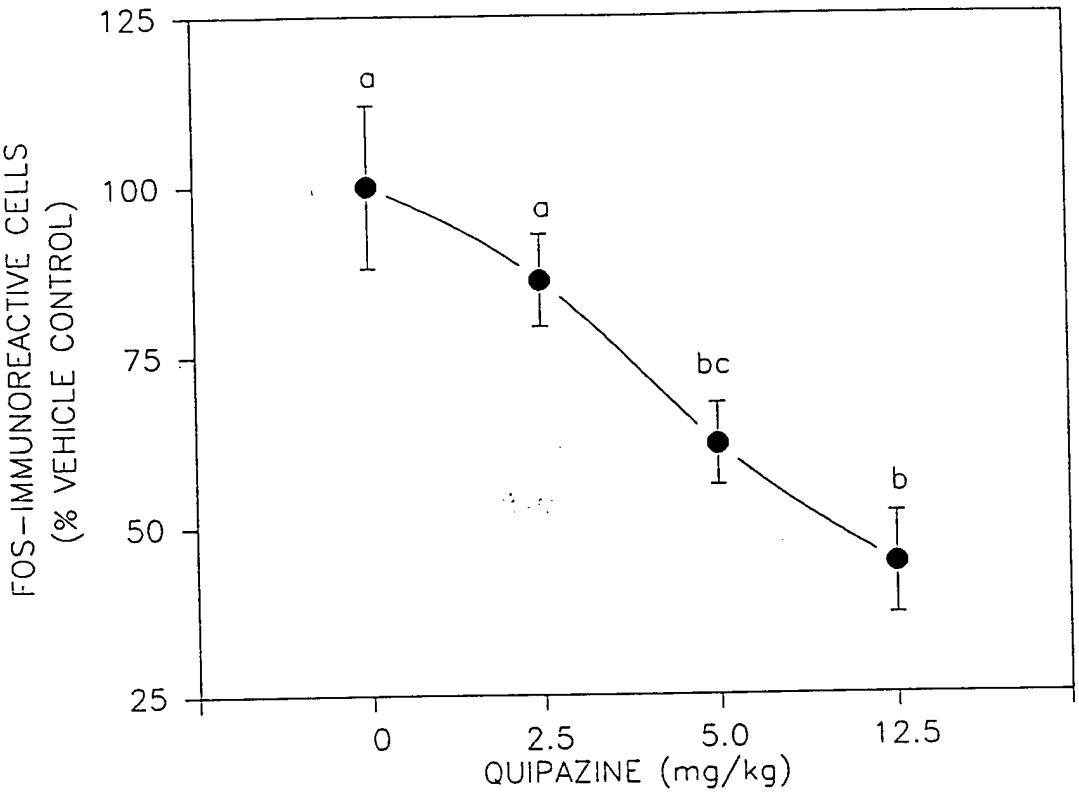
Effects of quipazine pretreatment on light-induced Fos expression at ZT 19

Pretreatment with an i.p. injection of the non-selective serotonergic, quipazine significantly suppressed light-induced Fos-LI in the SCN in a dose-dependent manner (Figure 4C,D and 5). The highest dose of quipazine used (12.5 mg/kg) caused a 55.6 $\pm$ 7.5% reduction in the number of immunoreactive cells ($p<0.05$ vs. vehicle controls). The effect of this treatment was anatomically selective since most Fos-LI in the ventrolateral zone of the SCN was abolished, while immunoreactivity in the lateral and dorsal regions persisted (Figure 4C,D). Pretreatment with lower doses of quipazine (5 and 2.5 mg/kg) caused a 38.1 $\pm$ 6.0% and 13.8 $\pm$ 6.8% reduction in SCN stained cells, respectively ($p<0.05$ and $p>0.4$, respectively). Quipazine treatment did not affect Fos-LI

Figure 4. A & B: Representative photomicrographs of light-induced Fos-LI in the SCN of vehicle-treated (control) hamsters. OC = optic chiasm. C & D: Photomicrographs of light-induced Fos-Li in the SCN of hamsters pretreated with an i.p. injection of the non-selective serotonergic, quipazine, (12.5 mg/kg). Note lack of immunoreactive cells in the ventrolateral SCN region. Calibration bar = 120 um.


A
B
C
D

Figure 5. Dose-response relationship between quipazine pretreatment and photic induction of Fos expression in the SCN. Points with different letters are significantly different, $p<0.05$. Vertical bars represent S.E.M.


125
100
75
50
25
FOS-IMMUNOREACTIVE CELLS
(% VEHICLE CONTROL)
a
a
bc
b
0
2.5
5.0
12.5
QUIPAZINE (mg/kg)

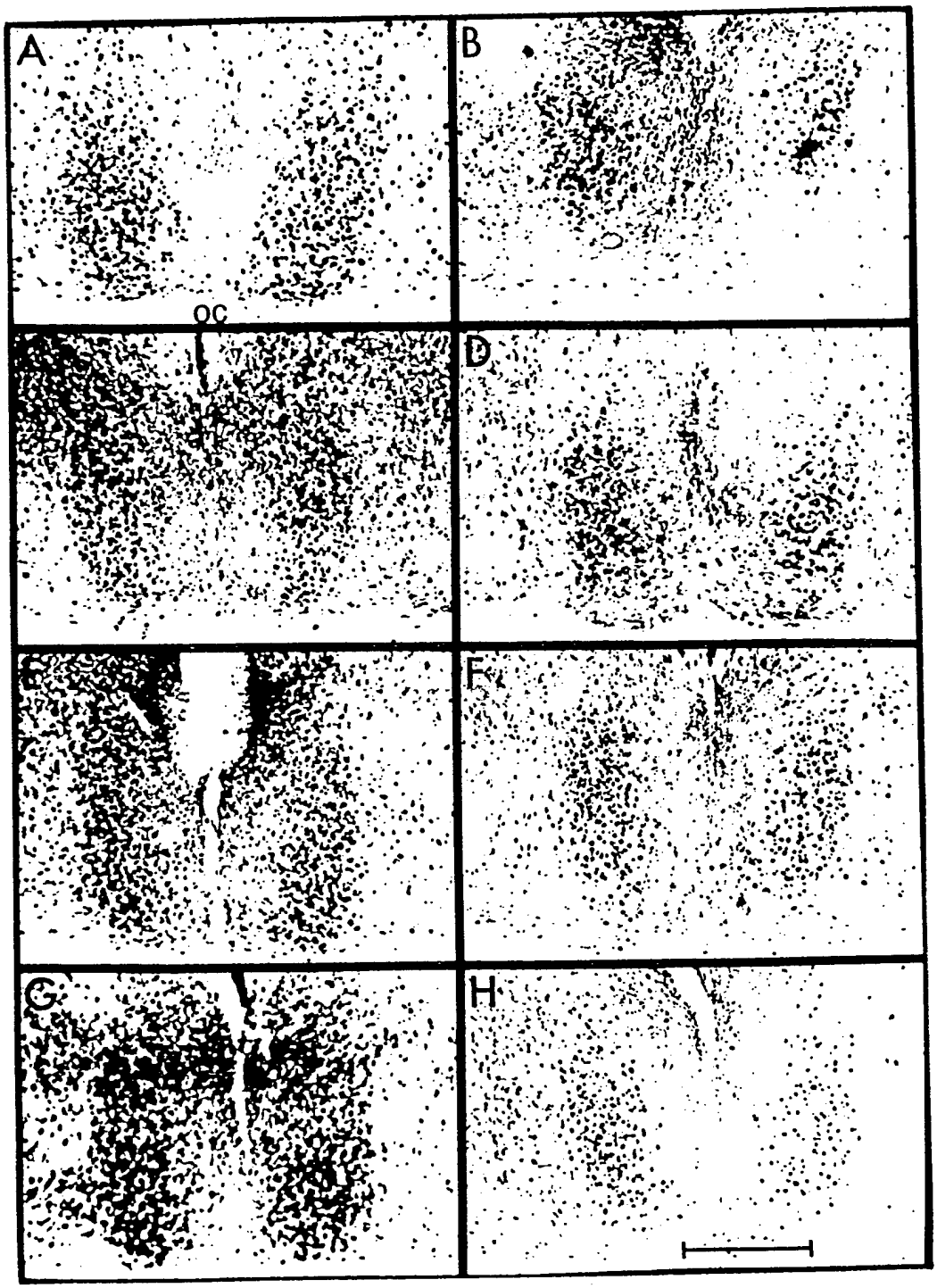
expression in the periventricular nuclei, SON or other brain regions.

Experiment 2:

Effects of 5-HT_{1A} and 5-HT_{1B} receptor agonists on light-induced Fos-LI during the late subjective night (ZT 19)

Pretreatment with the 5-HT_{1A} receptor agonist, 8-OH-DPAT, caused a significant reduction in the number of SCN cells expressing Fos-LI in a dose-dependent manner (Figure 7 and 6B). Treatment with 1, 2.5 and 5 mg/kg of 8-OH-DPAT suppressed Fos-LI by 38.2 ± 12.6%, 45.7 ± 8.1% and 42.5 ± 2.7%, respectively (all $p<0.01$ vs. vehicle controls). The lowest dose of 8-OH-DPAT (0.25 mg/kg) had little suppressive effect (9.6 ± 8.1% suppression, $p>0.1$ vs. controls). This effect of 8-OH-DPAT was limited principally to the ventrolateral region of the SCN, with little effect in the dorsal and medial SCN regions. The inhibitory effect of 8-OH-DPAT on light-induced Fos-LI was blocked by prior administration of the 5-HT_{1A} receptor antagonist, NAN-190 (100.9 ± 6.0% vs. vehicle controls, $p>0.9$; Figures 6C,8). Pretreatment with NAN-190 alone had little effect on light-induced Fos-LI (100.4 ± 6.4% vs. vehicle controls, $p>0.9$; Figures 6E,8). Pretreatment with another 5-HT_{1A} receptor agonist, buspirone, caused a 43.0 ± 1.3% reduction in SCN labelled cells ($p<0.001$ vs. vehicle controls; Figures 6D,9).

Figure 6. Representative photomicrographs of coronal sections through the mid-caudal SCN region showing effects of various serotonergic agents on light-induced Fos-LI. A: vehicle control. B: pretreatment with the 5-HT_{1A} agonist, 8-OH-DPAT. C: abatement of 8-OH-DPAT effect with the 5-HT_{1A} antagonist, NAN-190. D: pretreatment with another 5-HT_{1A} agonist, buspirone. E: pretreatment with NAN-190 alone. F: pretreatment with L-tryptophan (150.0 mg/kg). G: treatment with the 5-HT_{1B} agonist, TFMPP. H: pretreatment with a mixture of L-tryptophan, harmaline and fenfluramine. Calibration bar = 250 μm.


A
B
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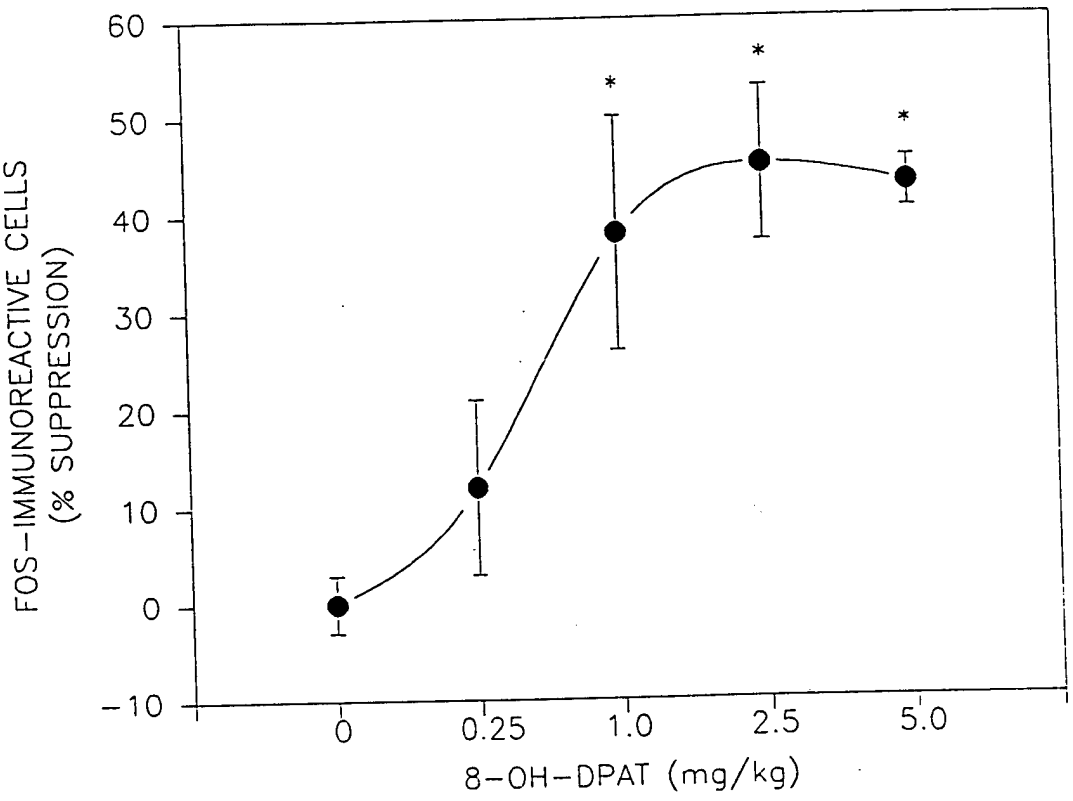
H

Figure 7. Dose-response relationship between 8-OH-DPAT and extent of suppression of light-induced Fos expression in the SCN. * Significantly different from vehicle-injected controls, $p<0.05$. Vertical bars are S.E.M.


60
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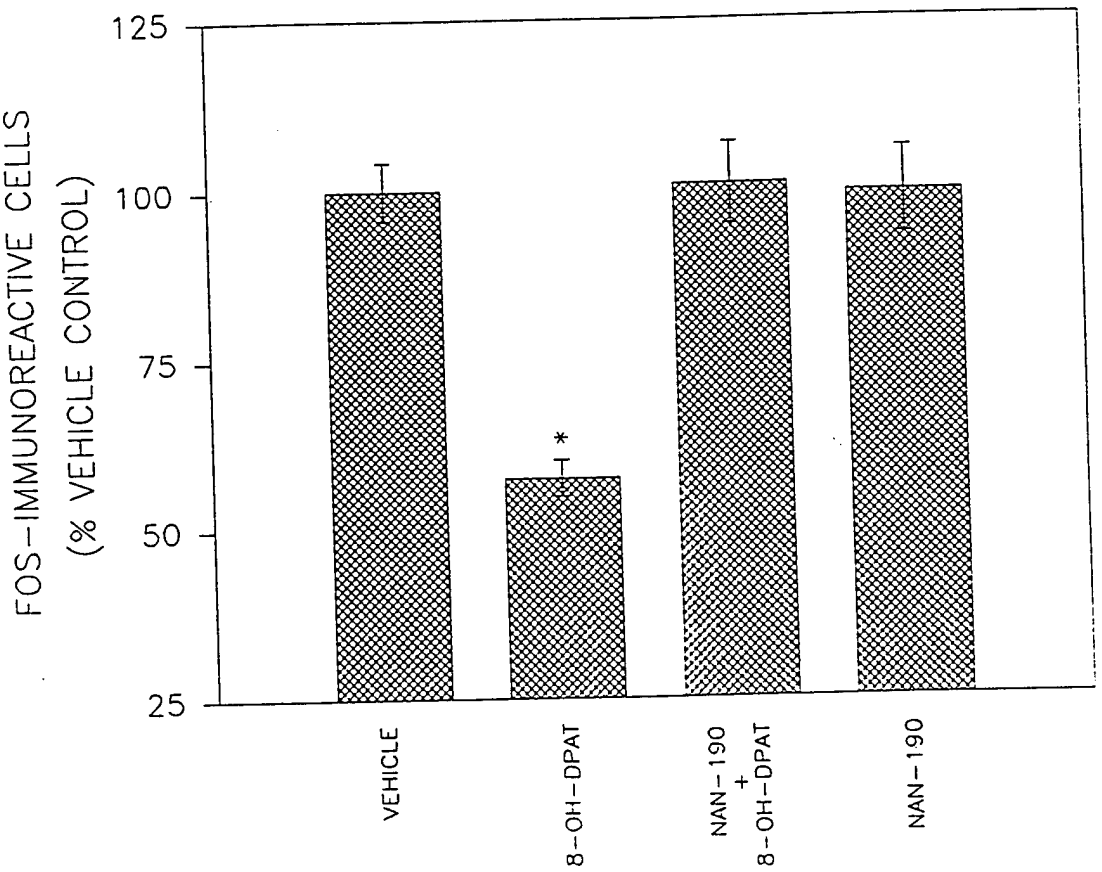
40
30
20
10
0
−10
FOS−IMMUNOREACTIVE CELLS
(% SUPPRESSION)
*
*
*
0
0.25
1.0
2.5
5.0
8−OH−DPAT (mg/kg)

Figure 8. Effect of 8-OH-DPAT (5.0 mg/kg) and/or NAN-190 on light-induced Fos-LI in the SCN. Points with different letters are significantly different, $p<0.05$. Vertical lines represent S.E.M.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(% VEHICLE CONTROL)
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25
*
VEHICLE
8-OH-DPAT
NAN-190
+
8-OH-DPAT
NAN-190

Pretreatment with the 5-HT_{1B} receptor agonist, TFMPP, resulted in a small but significant inhibition of light-induced Fos-LI (14.8 ± 3.5% suppression, $p<0.05$ vs. vehicle controls; Figures 6G,9).

Effects of 5-HT_2 and 5-HT_3 receptor agonists on Fos-LI

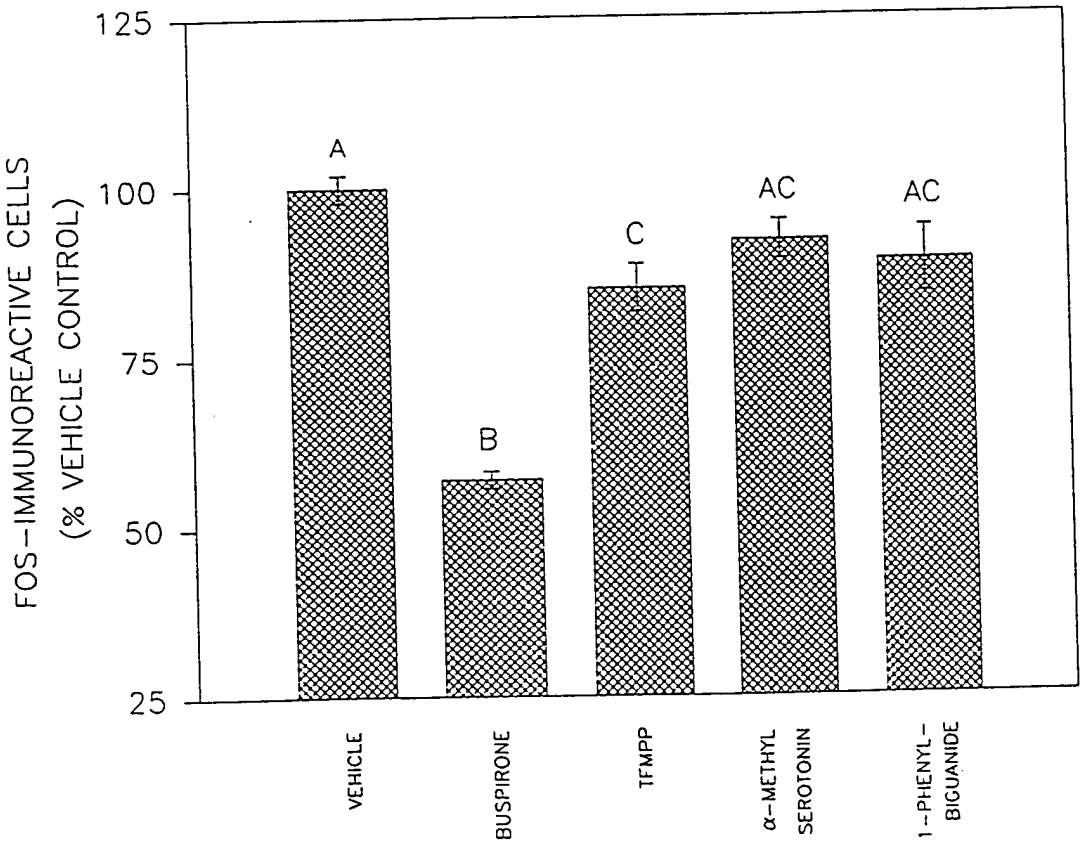
Pretreatment with the 5-HT_2 or 5-HT_3 receptor agonists, α-methylserotonin and 1-phenylbiguanide, respectively, had little suppressive effect on light-induced Fos-LI in the SCN (7.9 ± 2.1% and 13.0 ± 5.0% suppression, both $p>0.1$ vs. vehicle controls; Figure 9).

Experiment 3:

Effects of enhanced *in vivo* 5-HT release on light-induced Fos-LI

Pretreatment with a mixture of the immediate 5-HT precursor, L-tryptophan, the 5-HT releaser, fenfluramine and the type A monoamine oxidase inhibitor, harmaline, caused a significant suppression of light-induced Fos-LI (53.2 ± 4.8% suppression, $p<0.01$ vs. vehicle controls; Figure 6H,10). Pretreatment with L-tryptophan alone also suppressed photic induction of Fos-LI in a dose-dependent manner (figure 6F,12). Treatment with 100, 150 and 200 mg/kg L-tryptophan suppressed Fos-LI by 35.68 ± 8.44%, 33.8 ± 4.5% and 76.96 ± 4.02%, respectively (all $p<0.01$ vs. vehicle controls). The

Figure 9. Effects of 5-HT receptor agonists on photic induction of Fos-LI in the SCN. Buspirone, 5-HT_{1A} agonist; TFMPP, 5-HT_{1B} agonist; α-methylserotonin, 5-HT_2 agonist; 1-phenylbiguanide, 5-HT_3 agonist. Vertical lines are S.E.M. Bars with different letters are significantly different, $p<0.05$.


125
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FOS-IMMUNOREACTIVE CELLS
(% VEHICLE CONTROL)
A
B
C
AC
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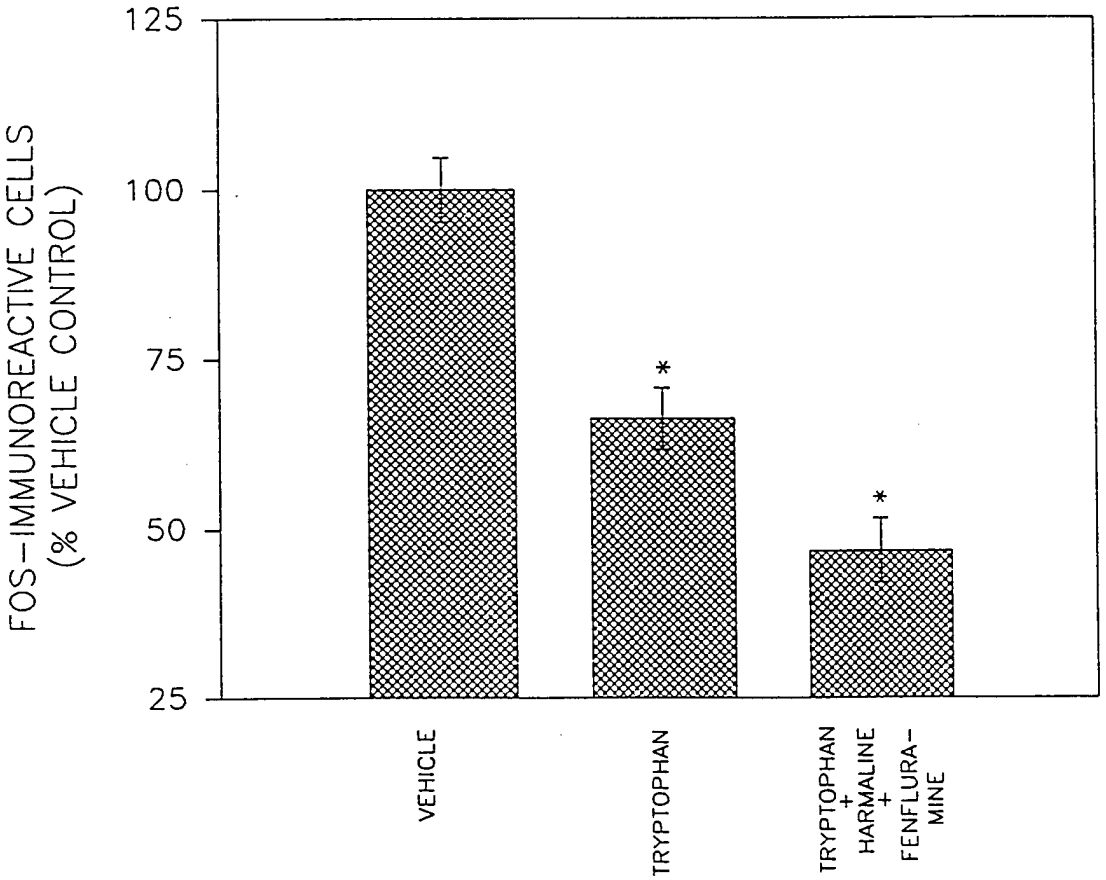
VEHICLE
BUSPIRONE
TFMPP
α-METHYL SEROTONIN
1-PHENYL-BIGUANIDE

Figure 10. Effects of pretreatment with L-tryptophan (150 mg/kg) alone or in combination with harmaline (type A MAO inhibitor) and fenfluramine (5-HT uptake blocker) on light-induced Fos-LI. * $p<0.05$ vs. vehicle controls. Vertical lines represent S.E.M.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TRYPTOPHAN
TRYPTOPHAN + HARMALINE + FENFLURA-MINE

inhibitory effect of these treatments was limited principally to the ventrolateral region of the SCN. The lowest dose of tryptophan (50 mg/kg) had little effect (3.42 ± 5.66% suppression, p>0.6 vs. vehicle controls).

Effects of 5-HT receptor antagonists on tryptophan-loading induced suppression of Fos-LI

Pretreatment with the 5-HT_{1A} receptor antagonist, NAN-190, prior to tryptophan-loading (200 mg/kg) caused a partial but significant reduction in tryptophan-induced suppression of light-induced Fos-LI (42.78 ± 6.68% suppression vs. 76.96 ± 4.02% in tryptophan-treated animals, p<0.01; Figures 11C,13). In contrast, tryptophan-induced suppression of Fos-LI was completely antagonized by pretreatment with either the non-selective 5-HT receptor antagonist, metergoline (109.27 ± 6.08% vs. tryptophan-treated animals, p<0.01; Figures 11D, 12) or the 5-HT_2/5-HT_{1C} receptor antagonist, ritanserin (5.96 ± 4.99% suppression vs. tryptophan-treated animals, p<0.01; Figures 11E,13). Neither metergoline (106.33 ± 4.34% vs. vehicle controls; p>0.8; Figure 11F,13) nor ritanserin (101.16 ± 5.54% vs. vehicle controls, p>0.8; Figure 11G,13) by itself had a significant effect on light-induced Fos-LI.

Experiment 4:

Effects of melatonin on light-induced Fos-LI

Pretreatment with melatonin (1 mg/kg) had little effect

Figure 11. Photomicrographs of representative sections through the SCN of the Syrian hamster demonstrating the effects of pretreatment with L-tryptophan alone or in combination with various serotonergic antagonists on light pulse-induced Fos-LI. A: vehicle control. B: L-tryptophan alone (200 mg/kg). C: L-tryptophan + NAN-190. D: L-tryptophan + metergoline. E: L-tryptophan + ritanserin. Note the partial suppression of L-tryptophan effect with NAN-190 in (C) and the complete attenuation of its effect in (D) and (E). F: pretreatment with metergoline alone. G: ritanserin alone. H: effect of pretreatment with melatonin. Calibration bar = 250 μm.

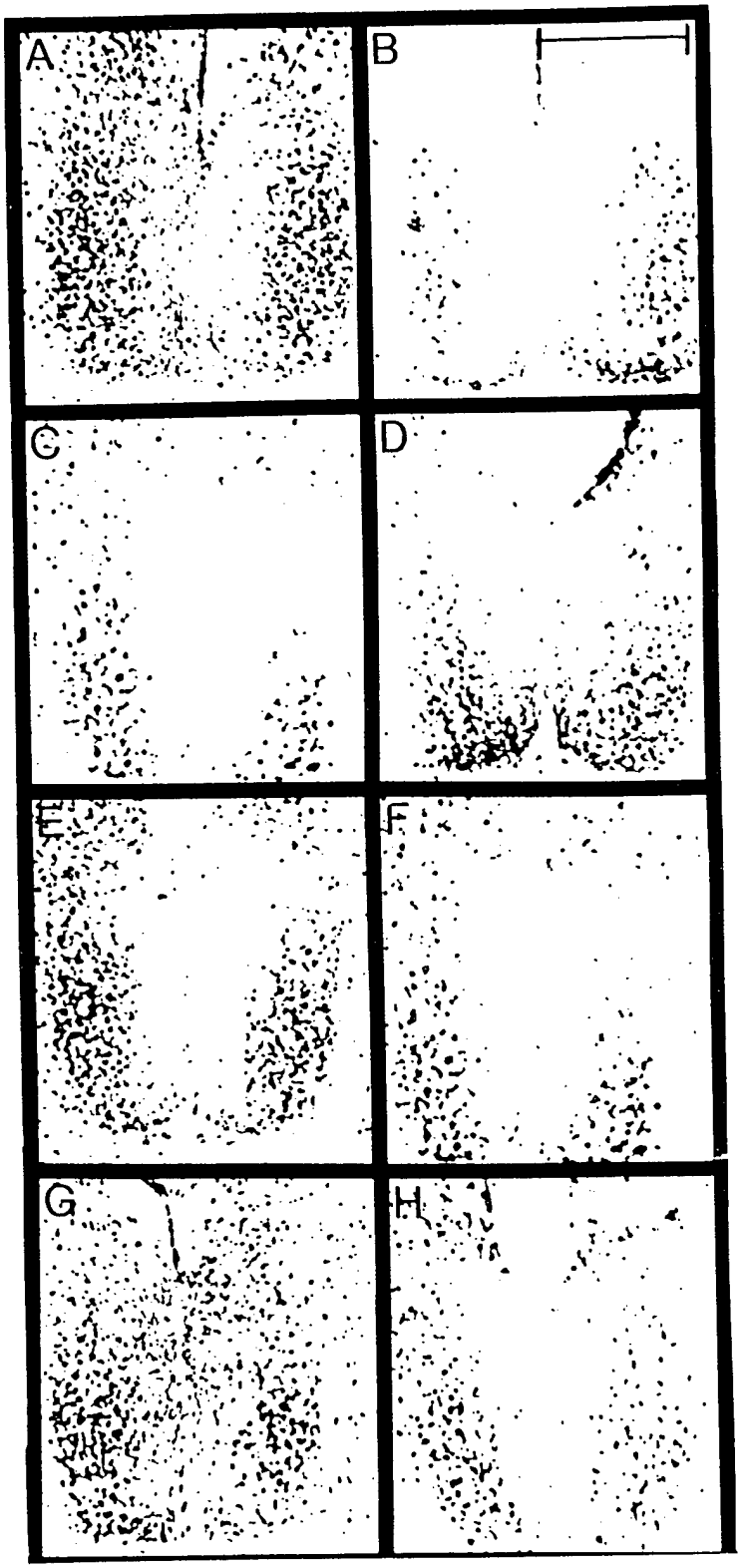

A
B
C
D
E
F
G
H

Figure 12. Dose-response relationship between L-tryptophan (i.p.) and extent of inhibition of light-induced Fos-LI in the SCN. * Significantly different from vehicle-treated controls, $p<0.05$. Vertical bars represent S.E.M.

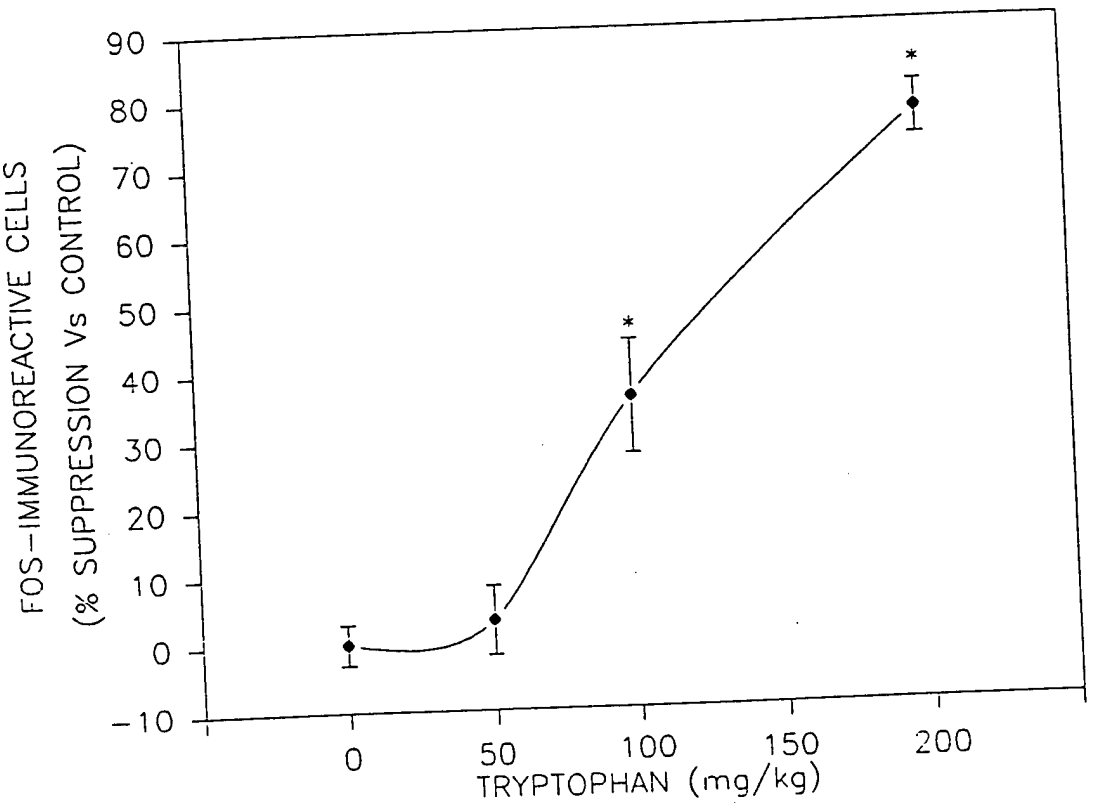
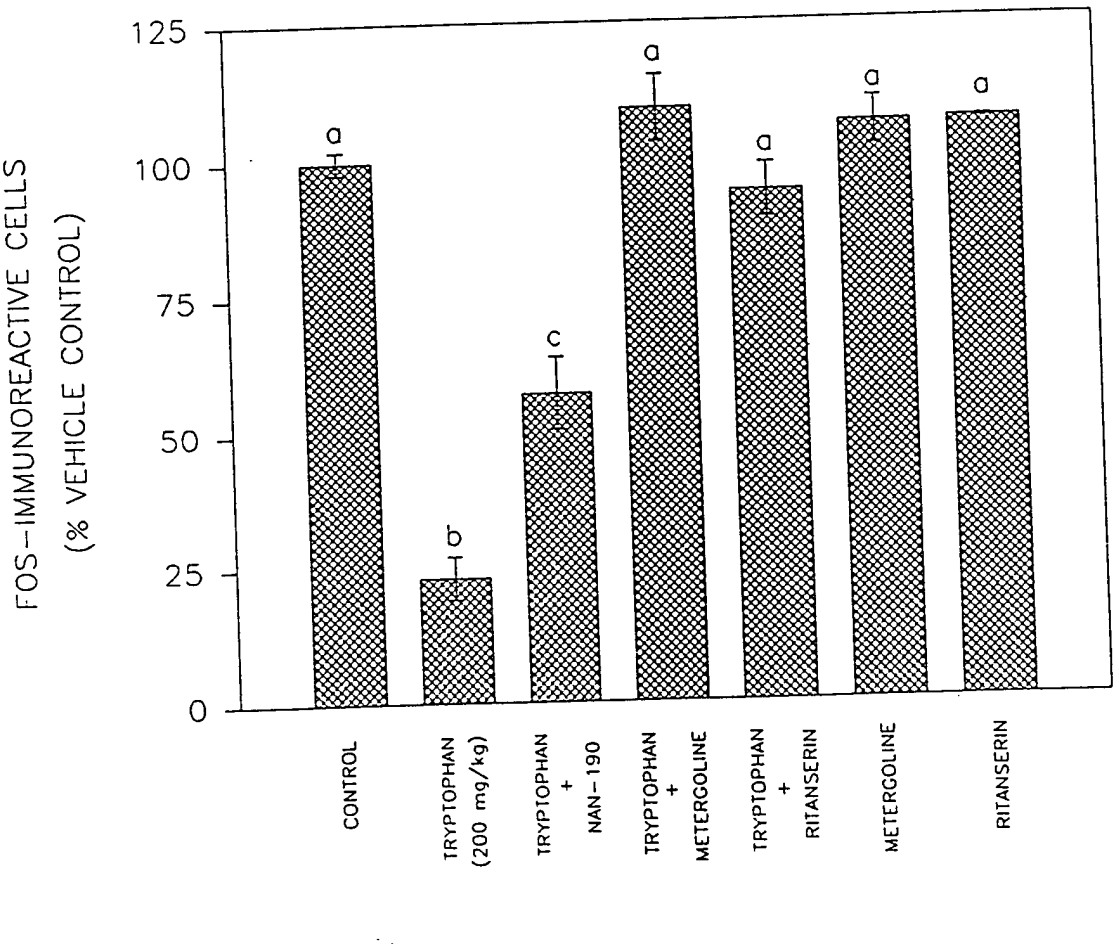

FOS-IMMUNOREACTIVE CELLS
(% SUPPRESSION Vs CONTROL)
90
80
70
60
50
40
30
20
10
0
-10
0
50
100
150
200
TRYPTOPHAN (mg/kg)
*
*
*

Figure 13. Effects of L-tryptophan (200 mg/kg) and/or 5-HT receptor antagonists on light-induced Fos-LI in the SCN. Metergoline, non-selective 5-HT antagonist; ritanserin, 5-$HT_{2/1C}$ antagonist; NAN-190, 5-HT_{1A} antagonist. Bars with different letters are significantly different, $p<0.05$. Vertical lines are S.E.M.


FOS-IMMUNOREACTIVE CELLS
(% VEHICLE CONTROL)
125
100
75
50
25
0
a
b
c
a
a
a
a
CONTROL
TRYPTOPHAN (200 mg/kg)
TRYPTOPHAN + NAN-190
TRYPTOPHAN + METERGOLINE
TRYPTOPHAN + RITANSERIN
METERGOLINE
RITANSERIN

on light-induced Fos-LI during the late subjective night(104.78 ± 8.73% vs. vehicle controls; $p>0.8$; Figures 11H,13).

Experiment 5:

Effects of tryptophan-loading on locomotor activity rhythms

Animals treated with L-tryptophan prior to lights-on, exhibited a significant delay in their activity onset the next night (36.0 ± 8.0 minutes, $p<0.05$ vs. vehicle controls; Figure 15). This phase-delaying effect of tryptophan on light-entrained activity rhythm was reversed following cessation of tryptophan injections.

Experiment 6:

Effects of light stimulation on Fos expression during the early subjective night (ZT 13)

Exposure of the hamsters to a 30 minute-duration light pulse (120 lux) delivered at ZT 13 did not induce Fos-LI in the SCN of any of the vehicle-treated animals (data not shown). Scattered immunoreactive cells were observed in other parts of the brain, such as the SON, PVN, piriform cortex and thalamus.

Effects of serotonergic agonists and/or antagonists on photic induction of Fos-LI during the early subjective night

Pretreatment with either quipazine or NAN-190 failed to induce Fos-LI in the SCN in response to a phase-delaying

on light-induced Fos-LI during the late subjective night(104.78 ± 8.73% vs. vehicle controls; $p>0.8$; Figures 11H,13).

Experiment 5:

Effects of tryptophan-loading on locomotor activity rhythms

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Effects of serotonergic agonists and/or antagonists on photic induction of Fos-LI during the early subjective night

Pretreatment with either quipazine or NAN-190 failed to induce Fos-LI in the SCN in response to a phase-delaying

Figure 14. Effects of pretreatment with melatonin on photic induction of Fos-LI in the SCN. Bars with different letters are significantly different, $P<0.05$. Vertical lines are S.E.M.

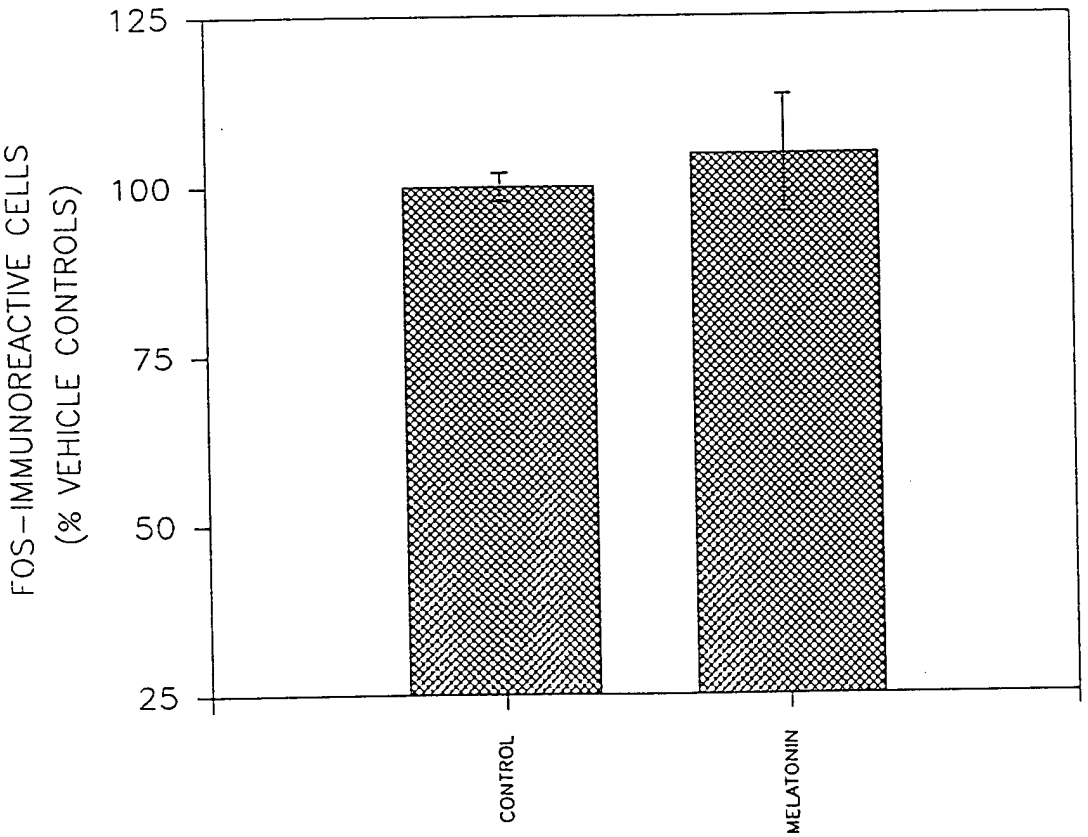
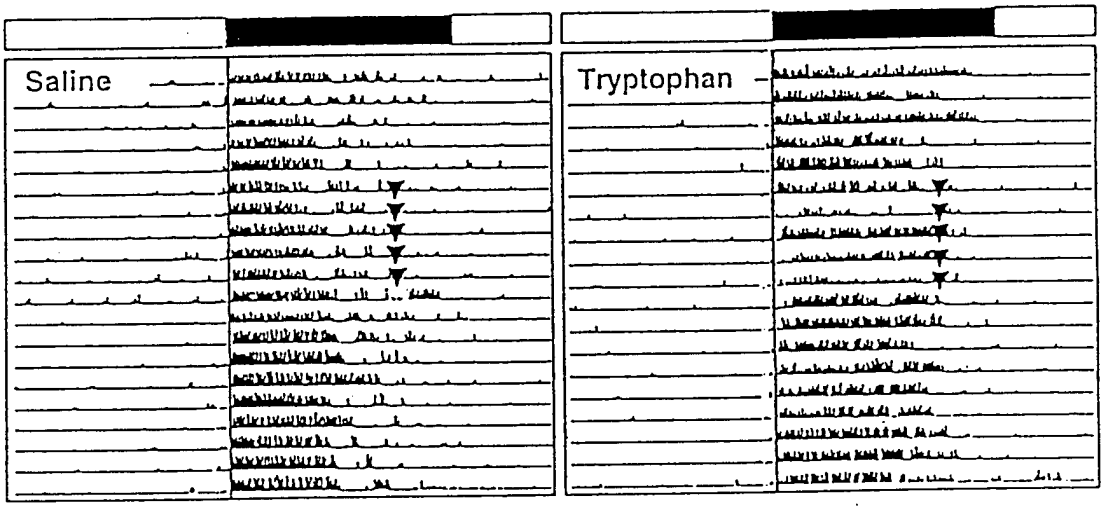

FOS-IMMUNOREACTIVE CELLS
(% VEHICLE CONTROLS)
125
100
75
50
25
CONTROL
MELATONIN

Figure 15. Double plot of behavioral wheel-running activity rhythms before and after treatment with L-tryptophan (150 mg/kg) at ZT 21 (60 minutes before lights-on) for 5 consecutive days. Days and times of injections are indicated by triangles. Little but significant ($p<0.05$) phase delay occured following treatment and was reversed after its cessation.


Saline
Tryptophan

light pulse. Pretreatment with metergoline resulted in a few faintly-stained cells localized to the SCN, which precluded analysis of drug action like that undertaken in the previous experiments.

EXECUTIVE SUMMARY

Contents:

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